Remarks

Status of Claims

Pending claims 11 and 23 stand as finally rejected. Independent claim 11 is directed to an isolated protein comprising the amino acid sequence set forth in SEQ.ID.NO.:12. Dependent claim 12 recites that the protein consists of the amino acid sequence set forth in SEQ.ID.NO:12. Applicant respectfully requests favorable reconsideration of these claims in view of the following remarks.

The Claims Meet the Utility and Enablement Requirements

In the final Office Action, the Examiner maintained the rejection of claims 11 and 23 under 35 U.S.C. § 101 for lack of utility, and similarly the rejection under 35 U.S.C. § 112, first paragraph, for lack of enablement. For the reasons given in the remarks included in Applicant's previous response filed March 23, 2005, which for the sake of brevity are incorporated by reference herein, as supplemented by the remarks below, these rejections are in error and should be withdrawn.

As noted previously, in determining whether the utility requirement has been met, the proper presumption is that Applicant's statement of utility is correct. See, e.g., In re Langer, 503 F.2d 1380, 183 U.S.P.Q. 288 (CCPA 1965). The Examiner still has not cited any literature to shore up the argument that artisans would doubt Applicant's stated utility. On the other hand, even though the burden has not been properly shifted to Applicant, to support the credibility of the asserted utility of the claimed invention Applicant submits herewith a review-type article by Benham et al., entitled "TRPV channels as temperature sensors", *Cell Calcium* **33** (2003): 479-487 (Exhibit A).

The Benham et al. article demonstrates the acceptance in the art of the biological function of TRPV family members in heat sensing and notes the importance of such a role in animals. This article reflects that artisans would find credible the disclosed biological function of the claimed protein in heat response discovered by Applicant in light of the description in the present specification. Accordingly, the rejections are untenable.

Conclusion

For the foregoing reasons, the claimed invention is supported by a specific, substantial, and credible utility. Consequently, the final rejections under Section 101 and the first paragraph of Section 112 should be withdrawn.

Respectfully submitted,

Date: September 5, 2005

Linda S. Evans
Reg. No. 33,873

Exhibit A

CHURCHILL LIVINGSTONE

Cell Calcium 33 (2003) 479–487

cell calcium

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TRPV channels as temperature sensors

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Received 5 February 2003; accepted 10 February 2003

Abstract

The past year has seen a doubling in the number of heat-sensitive ion channels to six, and four of these channels are from the TRPV family. These channels characteristically have Q_{10} values of >10 above the thermal threshold, very different from the Q_{10} values of 1.5–2.0 seen in most ion channels. Cells expressing TRPV1 show similar temperature sensitivity to small capsaicin-sensitive nociceptor neurons, consistent with these neurons expressing homomers of TRPV1. A-δ fibres exhibit properties that may be explained by TRPV2 containing channels which is present in large diameter sensory neurons that do not express TRPV1. TRPV3 has a lower temperature threshold and may contribute to warm-sensitive channels together with TRPV1. Warm sensation may also be transduced by TRPV4 expressing sensory neurons and hypothalamic neurons. We can now look forward to further work defining the properties of the recombinant channels in more detail and a re-analysis of endogenous i_{heat} currents in thermosensitive neurons and other cells. Data from the study of mice in which TRPV2, TRPV3 or TRPV4 have been deleted are also eagerly awaited.

Keywords: TRPV channels; Thermosensor; Neuron

1. Introduction

Temperature sensing is important in all animals. Mammals require precise assessment of body temperature for setting internal thermoregulation, while cold-blooded animals need to sense internal body temperature and to sense warm and cool surroundings to regulate their behaviour in seeking warming or cooling environments. In addition, all animals depend on the rapid sensation of noxious heat to activate rapid avoidance reflexes.

In principle, sensitivity to small temperature changes could be conferred by incorporating any biological reaction with high entropy into a signal transduction cascade. In *Caenorhabditis elegans* there is evidence that levels of cGMP in a sensory neuron confer thermosensation through their gating of *tax4* the *C. elegans* analogue of the mammalian cyclic nucleotide gated channel [1]. In mammals also, ion channels seem to be the main signal transduction mechanism for thermosensation, but at least some of these mammalian channels are directly gated by heat. The properties of all ion channels are affected by temperature but effects are modest, usually resulting in small linear increases in current flow with Q_{10} values of around 2, where Q_{10} is the change in rate of the reaction resulting from a 10 °C rise in temperature. However, the heat-sensitive channels are characterised by having gating mechanisms that show a much greater sensitivity for heat than standard biochemical reactions and have Q_{10} values much greater than 2.

Of the six molecularly defined heat-sensitive channels, one is a two-pore domain potassium channel, TREK-1 [2] while the other five are all cation channels from the TRP family. The TRPV or vanilloid sub-family, currently comprises six members, four of which sense temperatures around and above body temperature. The fifth temperature-sensitive TRP channel is TRPM8 [3,4] from the melastatin sub-family which functions as a cold sensor, responding to decreases in temperature below 22 °C. This review summarises recent work on the temperature sensitivity of the TRPV family of ion channels and attempts to correlate these properties with endogenous temperature-sensitive currents in native tissues.

2. Properties of recombinant heat-sensitive TRPV channels

The TRPV channels are a sub-group of the TRP family of cation channels [5,6]. Structurally, these channels share

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doi:10.1016/S0143-4160(03)00063-0

homology with potassium channels. Each protein sub-unit has six trans-membrane domains and recent experimental work confirms the expectation based on analogy to potassium channels that the functional channels are likely composed of tetramers [7]. Homologous genes have been identified in *C. elegans*, *osm-9* is the most closely related gene to mammalian TRPV1. Interestingly, *osm-9* gene product functions in *C. elegans* as an osmosensor and plays no part in thermosensation which is performed by a single thermosensitive sensory neuron, the AFD cell [1]. A recent functional characterisation of two of four further TRPV homologues in *C. elegans* indicated that they too functioned as part of the osmosensing pathway [8]. Two open reading frames of genes that fall within the TRPV family have been identified in the *Drosophila* genome [8] but their function is not known. The exquisite temperature sensitivity of the mammalian TRPVs reviewed here may thus be an adaptation during the more recent evolution of warm-blooded animals.

Assessing the temperature sensitivity of an ion channel may be approached in an analogous manner to characterising its voltage dependent properties. The steady state properties of the current at different test potentials can be examined using an instantaneous voltage clamp or alternatively, a voltage ramp may be applied to rapidly determine the current flow at a range of potentials. Clearly, the latter is most useful when channels show little in the way of time dependent changes in gating at different potentials. Both temperature ramps and temperature jumps have been used to measure thermal sensitivity (cf. [9,10]) but not usually both techniques in the same laboratory. As the TRPV channels show slow time dependent temperature responses these properties will affect the data generated by the two stimulus protocols. This should be borne in mind when comparing properties of the channels listed in Table 1.

3. TRPV1

The expression cloning of the capsaicin-sensitive vanilloid receptor was a ground breaking landmark [11] from which subsequent work on the molecular basis of the other temperature-sensitive TRP channels followed. TRPV1 is a Ca^{2+} permeable cation channel activated by exogenous vanilloids such as capsaicin, but also by endogenous lipid signalling molecules such as anandamide [12] and eicosanoids [13]. As suspected, given the co-location of vanilloid sensitivity and noxious heat gated currents in small sensory neurons and the knowledge that capsaicin evokes a "hot" sensation in humans, VR1 or TRPV1 can be activated by noxious heat with a threshold of about 43 °C in rat [11,14] human [15] or by inference in the mouse knockout [16,17]. Rapid temperature jumps show that TRPV1 is activated relatively rapidly with currents reaching a plateau after less than 500 ms [15]. Thus, temperature ramps of $<0.5\,°C\,s^{-1}$ will report currents close to the steady state values. This may explain the consistent responses reported above, obtained from ramps or temperature jumps.

The temperature threshold for TRPV1 is not fixed but modulated by chemical ligands and the phosphorylation state of the channel. The various activating ligands have synergistic effects, so that any specific chemical ligand concentration will result in a unique setting of the temperature sensitivity of the channel. Thus, changes in endogenous lipid ligand concentration might be expected to vary the thermal sensitivity of the channels. The phosphorylation state of the channels is also important. So, for example, phosphorylation of TRPV1 by protein kinase C results in activation of the channel at normal body temperature [18]. This plasticity potentially confers a broad range of temperature sensitivity to cells expressing TRPV1. Thus, while responses in recombinant expression systems such as HEK293 cells in standard culture conditions may be quite consistent, there is much

Table 1
Properties of i_{heat} in recombinant systems expressing TRPV subunits

Channel expressed	TRPV1	TRPV2	TRPV3	TRPV4
Pseudonyms	VR1	VRL-1	VRL-3	VRL-2, VR-OAC, OTRPC4, trp12
High expression	DRG, TG	DRG, TG	DRG, TG, skin	TG
TRPV heteromers	1 and 3	Not 2 and 1	3 and 1	?
p_{Ca}/p_{Na}	9.6	2.9	12.1	6.3, 4-αPPD
i_{heat}			2.6	
Heat threshold (°C)	>43	>53	>31	>24
			>35	>33
			>39	
Q_{10}	21	?	25 or 6.6	10 or 19
Effect of prior heating	Sensitises/desensitises	Strongly sensitises	Strongly sensitises	Desensitises
Threshold shift after pre-heating (°C)	~−7	−13	−4	+6
i_{heat} in isolated patches	Yes	Not tested	Yes	No
$[Ca^{2+}]_i$	Desensitises	?	Desensitises	Blocks IC_{50} 0.4 μM
Ruthenium red	Blocks	IC_{50} 0.6 μM	IC_{50} < 1 μM	Voltage dep block
Capsazepine	Blocks	Inactive	Inactive	Inactive

more potential for a range of responses in native cells. This must be borne in mind when attempting to explain native currents in molecular terms.

4. TRPV2

An initial search of genomic databases for TRPV1 homologues yielded vanilloid receptor like protein 1 or VRL-1, now called TRPV2, that is expressed in a sub-population of medium to large sensory neurons but also at lower levels in other tissues. This family member was not activated by any TRPV1 chemical ligand, but was activated by noxious heat >53 °C resulting in a cation current that was Ca^{2+} permeable. The current showed outward rectification at positive potentials, like other TRP channels, but also inward rectification at very negative membrane potentials. The temperature-evoked currents were specific to TRPV2 transfected cells whether oocytes or HEK293 cells, and potently blocked by ruthenium red (IC_{50} 0.6 μM) consistent with current flow through a specific ion channel pathway. Repeated heating resulted in sensitisation such that the current threshold moved to much lower temperature at around 40 °C [19].

This initial description provides a clear role for TRPV2 channels in sensing high threshold noxious heat. The broader distribution suggests other possible functions. Interestingly, the murine form of TRPV2 was shown to be constitutively active at room temperature following treatment of TRPV2 transfected CHO cells with insulin-like growth factor (IGF-1) for a few minutes. The development of a functional cation current correlated with the IGF-1 stimulated translocation of the channel protein to the cell membrane. The mouse isoform (79% homology to rat TRPV2 was also sensitive to noxious heat, currents being increased by 140% compared to currents at room temperature [20].

Attempts by several laboratories including our own to measure temperature gated currents in TRPV2 transfected cells have been unsuccessful, indicating that important aspects of the functional expression of this channel remain to be determined. The recent suggestion that soluble co-factors are required to mediate heat responsiveness of TRPV4 (see below) may be a clue to the variable success in evoking heat gated currents from TRPV2 expressing cells.

5. TRPV3

Analysis of thermosensation in the TRPV1 null mouse demonstrated virtually normal thermal nociception in the absence of inflammation [16,17]. Sensing hot temperatures could be ascribed to TRPV2 but there was clearly a need for further molecular candidates for warm sensation in addition to TRPV1. The virtual completion of the human genome project provided genomic sequence in which to search for any outstanding TRPV homologues. This search yielded one further member, TRPV3, which is expressed mainly in the CNS and sensory neurons in humans [9,10], but also in skin and in particular in keratinocytes found at the inner boundary of the epidermis [21]. Applying temperature ramps to CHO or HEK293 cells expressing TRPV3, evoked a temperature-sensitive cation current with moderate permeability to Ca^{2+} and a high Q_{10} [10,21]. To date there is no evidence that TRPV3 can be activated by any chemical ligand. Heating isolated outside out patches from TRPV3 expressing cells activated a cation channel of 172 pS unitary conductance [22]. This suggests that direct heating of the channel protein or at least a membrane delimited pathway mediates channel opening.

Thermal sensitivity depended on the thermal history of the cell and this may be one reason why there is some variation in the reported threshold of activation of TRPV3, ranging from 23 °C [10] through 35 °C [21] to 39 °C [9]. Repeated warming sensitises the channel to heating, both increasing the maximum current at the end of the temperature ramp and shifting the temperature threshold to lower temperatures. The effect of repeated heating to 48 °C on currents recorded from TRPV1 and TRPV3 expressing HEK 293 cells is shown in Fig. 1.

All three groups used electrophysiological or Ca^{2+} fluorescence recording based on a baseline resting temperature at room temperature. While all cells will have been incubated at 37 °C post transfection, the duration held at room temperature before commencing recording probably varied and might be expected to affect the subsequent temperature sensitivity. Prolonged incubation at 37 °C might also select against cells expressing channels with low temperature thresholds. Whatever the precise thermal sensitivity of the channel, from a parallel comparison of TRPV1 and TRPV3, it appears that TRPV3 has a lower temperature threshold than TRPV1 [9].

A further complexity is the demonstration that TRPV3 can heteromise with TRPV1 when expressed in HEK293 cells. These heteromers may function with many of the polymodal properties of TRPV1 including capsaicin and proton sensitivity. Co-localisation in native DRG cell bodies suggests that this may happen in native cells [9]. Further careful comparison of TRPV1, TRPV3 and TRPV1/3 expressing cells will be needed to confirm that TRPV1/3 heteromers are functional and identify any differences from TRPV1 homomers. The profound differences between TRPV1 and TRPV3 behaviour (Fig. 1) may allow for heteromeric channels to be robustly identified and characterised.

6. TRPV4

TRPV4 is the final member of the TRPV family with reasonably close homology to TRPV1. TRPV5 and TRPV6, the ECAC channels are more distant cousins [6]. The TRPV4 channel was originally described as an osmosensor, opening in response to hypotonic swelling of the cell [23,24]. While

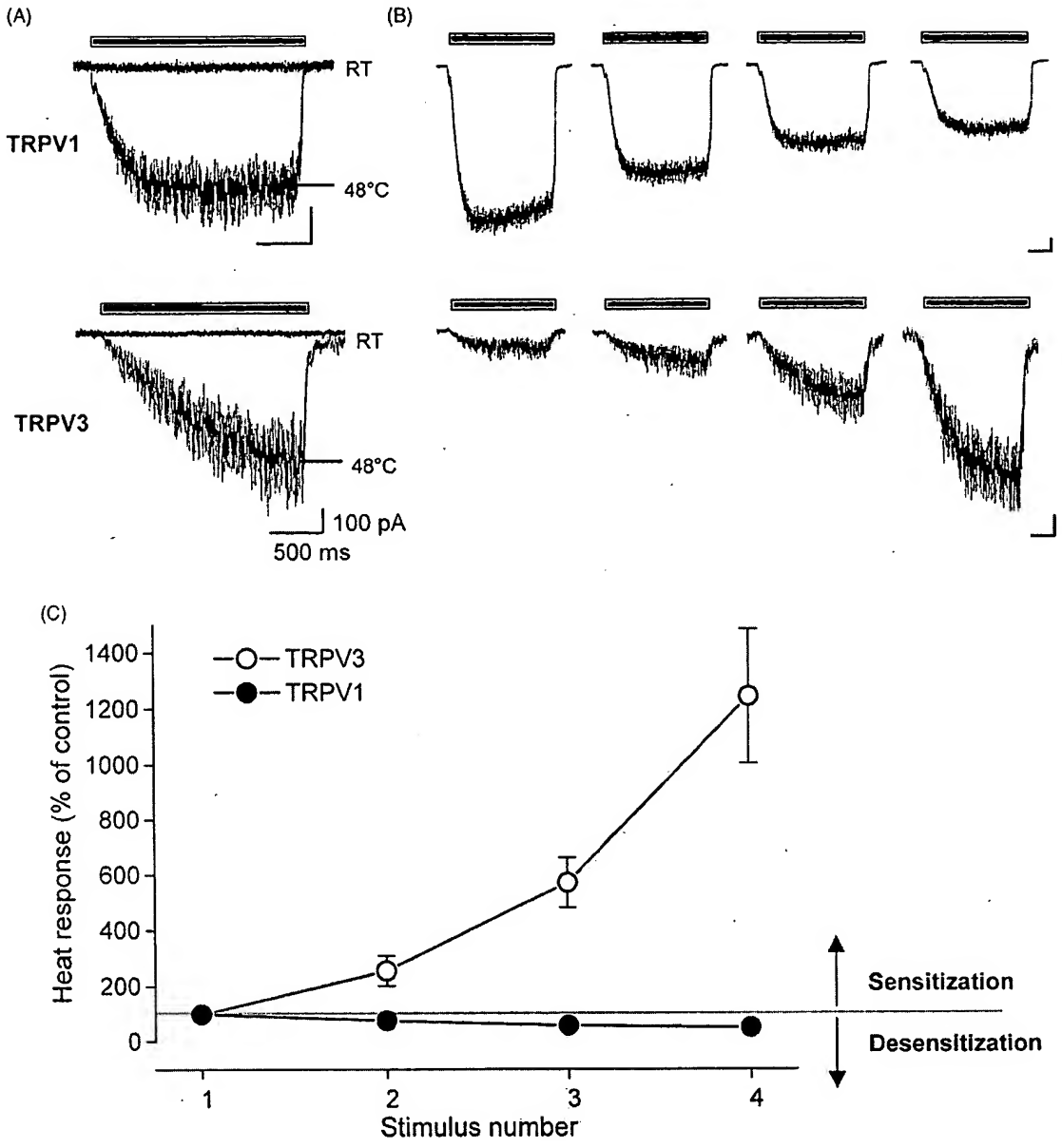

Fig. 1. Heat activation of TRPV1 and TRPV3. (A) Expression of either TRPV1 or TRPV3 receptors alone in HEK293 cells generates heat-activatable ion channels with heat thresholds in the warm (TRPV3) or noxious (TRPV1) temperature ranges. Whole-cell patch clamp recordings of membrane currents in response to heat application (48 °C, or at room temperature, RT), for the duration of the bar, are shown. These traces typify the slower kinetics of TRPV3 receptor activation relative to TRPV1 [9]. It is also noteworthy that the inward heat-gated currents are also accompanied by an increase in current noise (variance) which is consistent with the stochastic activation of an ion channel of relatively high single channel conductance in both cases. (B) TRPV1 receptors typically desensitise in response to agonist stimulation such as capsaicin or acid. Heat activation of TRPV1 appears to cause similar effects. Repetitive stimulation of TRPV1 with supra-threshold heat stimuli (48–51 °C) led to pronounced receptor desensitisation (even in the nominal absence of external Ca^{2+}) such that the magnitude of inward current responses after four test stimuli at approximately 1 min intervals were only 51% of the original control response. The behaviour of TRPV3 is quite different since TRPV3 responses actually increase with repeated stimulation at supra-threshold temperatures (43–47 °C), indicating a marked sensitization of this receptor by heat. TRPV3 responses increased by approximately 100% at each subsequent stimulus challenge such that current responses increased by about 10-fold (1246%) over the course of the experiment. The pooled datasets for TRPV1 ($n = 3$–6) and TRPV3 ($n = 4$) are shown in panel (C) and experimental conditions are as described previously [15,9] for TRPV1 and TRPV3, respectively). The scale bars used are calibrated as follows: vertical, 100 pA; horizontal, 500 ms.

the initial reports suggested that the channel could not be activated solely by a rise in temperature, it was reported that responses to osmotic stress increased significantly at body temperature compared to room temperature [24].

More detailed examination of the properties of TRPV4 expressing cells has shown that TRPV4 also acts as a thermosensor. Application of temperature ramps from 22 °C to oocytes or HEK293 cells expressing rat TRPV4 resulted in

rises in intracellular Ca^{2+} and inward currents with thresholds around 34 °C. [25]. Starting from a lower holding temperature of 14 °C, inward currents were activated with a threshold of 24 °C in HEK293 cells expressing mouse TRPV4. Careful comparison of the heat-evoked current with that activated in the same cells by the TRPV4 agonist, 4-αPDD [26], supported the conclusion that the heat activated current was through TRPV4 channels [27]. In contrast to TRPV1 [14] and TRPV3 [22] (Table 1), isolated patches that contained functional TRPV4 channels showed no current activation when exposed to increasing temperature. This suggests that additional soluble factors are required to mediate thermosensation. Either heating produces a soluble ligand or a soluble ligand is required as a co-activator [27].

Over the range 24–36 °C, current increased with a Q_{10} of 19.1 and showed dramatic desensitisation towards the peak of the ramp. A further manifestation of this property was that repeated heat ramps evoked smaller responses with higher thresholds [27] in contrast to the properties of the other TRPVs (Table 1). Temperature sensitivity that spans normal body temperature suggests that TRPV4 can respond to small changes in body temperature around 37 °C but the rapid desensitisation properties make extrapolating these results to a steady state 37 °C hazardous. However, acclimatising cells to mammalian body temperature and then increasing temperature with a ramp produced further increases in intracellular Ca^{2+} indicating that TRPV4 expressing cells can sense a change in temperature as seen in pyrexia [25].

It will be interesting to generate comparative data using the same protocols as in Fig. 1 for TRPV4 and for TRPV2. Data summarised in Table 1 suggests that TRPV4 shows strong desensitisation of responses after prior heating and during a single heat ramp [27], while TRPV2 shows strong sensitisation [19]. Detailed information on the activation kinetics of these two channels is awaited. This should permit a more comprehensive comparison with the properties of native currents and aid identification of further functional heteromers.

7. Properties of heat-sensitive currents in sensory neurons

7.1. In vivo single unit recording

In vivo recording of the activity of single nerve fibres in response to heating the skin has identified four types of thermosensor with specific temperature sensitivities (Fig. 2) (reviewed by [28]). Warming skin from around 30 °C, the skin temperature if the ambient temperature is 20 °C, first excites a population of unmyelinated c-fibres which convey a sensation of innocuous warmth (Fig. 2). This temperature sensitivity indicates a role for TRPV3 as suggested by Peier et al. [21] who believe that the localisation of this channel in keratinocytes at the inner surface of the epidermis where sensory nerve terminals terminate, is important to warm temperature signal transduction. They propose a signalling pathway that involves temperature sensation by the keratinocytes that then excite nerve terminals by the release of a transmitter such as ATP. This neatly explains the lack of warm temperature responsive sensory neurons in DRG isolated from $TRPV1^{-/-}$ mice [16,17]. However, this observation might be expected given the low number of warm-sensitive primary afferent fibres and the relatively small sample size in

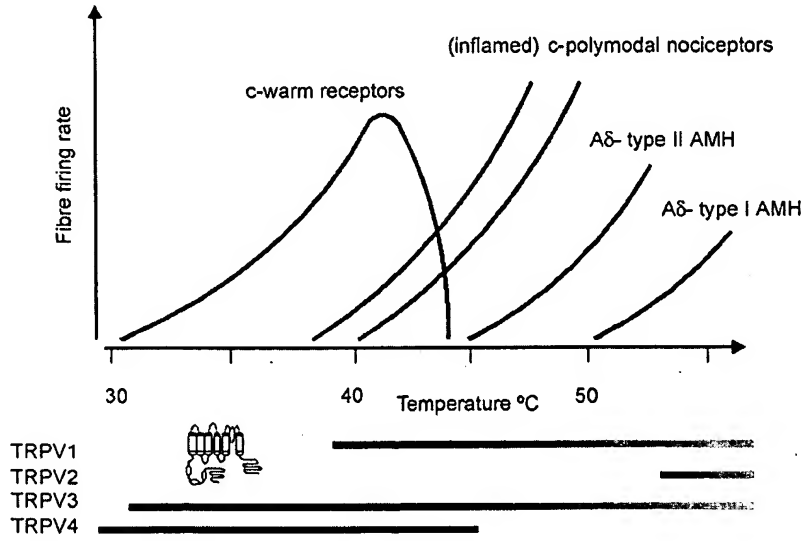

Fig. 2. Fibre firing rates plotted against temperature of the four types of thermosensors in somatic afferent nerves. For native fibres, temperatures reflect ambient temperature at the tissue surface, so that temperatures at nerve endings are likely to be a few degrees lower. C-polymodal nociceptors show leftward shifts in temperature threshold in response to inflammatory mediators (red line) as do TRPV1 expressing cells. Activation ranges of recombinant TRPV channels are indicated by the horizontal bars below *X*-axis. Note that there is some uncertainty over the activation thresholds for TRPV3 and TRPV4 (see Table 1).

Table 2
Properties of native i_{heat} recorded from rat dorsal root ganglion cells in culture

Classification [references]	Tissue	Cell diameter mean (μm)	Threshold (°C)	Q_{10}	P_{Ca}/P_{Na} i_{heat}	Desensitisation of heat response	Capsazepine block i_{heat}	Ruthenium red block IC_{50} (μM)
Low threshold capsaicin [30]	3–5 day rat DRG	<25	42	–	1.3	No		
sensitive cells [37]			43	17.8		Yes, decrease threshold		
[31], [33]	Adult rat DRG	18	45		1.2; $i_{capsaicin}$, 2.4		No effect	
[32]		27.5				No	IC_{50} 13 μM 25% insensitive	>5
Low threshold capsaicin insensitive [38]	3 day rat DRG	<20	40	>10		No, decrease threshold, biphasic, respectively		
LT capsaicin sensitivity n.d. [29]	Adult rat DRG	<30	40				No effect at 10 μM	No effect at 100 μM
High threshold [31]	Adult rat	25	51					
Capsaicin insensitive [34]	DRG	30	51.6		3.5		5 μM blocks 55% only	0.3

these studies, which may also explain why there is no detailed description of warm responsive sensory neurons in dissociated culture. Alternatively, culture conditions, age of neurons or the absence of satellite cells might also explain these observations in TRPV1$^{-/-}$ dissociated neutral cultures. Study of TRPV3$^{-/-}$ mice will be useful in further exploring the role of TRPV3, as will direct recordings of heat activated currents from isolated keratinocytes. The temperature range in which TRPV4 is activated also fits well with sensing warm temperatures, particularly the desensitisation properties as noxious temperatures are approached (Fig. 2). It is tempting to speculate that both channels may contribute in some sensory pathways.

As skin temperature is elevated beyond 40 °C, the firing rate of these c-fibres declines and a separate population of polymodal c-fibres are excited that are also responsive to noxious chemical and mechanical stimuli. This fibre phenotype correlates well with the behaviour of isolated small diameter sensory neurons that are responsive to capsaicin (see below and Table 2), suggesting a role for TRPV1 in thermosensation in these nerve endings. Further increases in temperature successively recruit myelinated A-δ fibres at thresholds of about 46 and 53 °C, the latter having a similar threshold to TRPV2. These types I and -II AMH fibres are also mechanosensitive resulting in the AMH (A, mechano and heat sensitive) nomenclature. In vitro correlates of these fibre phenotypes are seen in large diameter, capsaicin insensitive, sensory neurons, although clear distinction into two types is less obvious (see Table 2). Thus, these precise thermal sensitivities suggest that multiple thermosensitive sensory transduction elements are involved rather than for example, graded expression of a single temperature-sensitive element that might result in different temperature thresholds for firing, dependent on expression level.

7.2. *In vitro single cell studies*

Studies on sensory neurons in the late 1990s had suggested that there were multiple heat-sensitive channels contributing to noxious thermosensation. While small, capsaicin-sensitive, sensory neurons responded to temperatures >45 °C [29,30], larger diameter temperature-sensitive neurons, also distinguished by being capsaicin insensitive had temperature thresholds of around 51 °C [31].

The cloning of TRPV1 [11] and TRPV2 [19] rapidly provided a molecular basis for this diversity and immediately stimulated more detailed analysis of the properties of sensory neurons.

This recent work is summarised in Table 2. A number of studies have now provided many details of the properties of currents with activation threshold 40–45 °C that can be evoked in dorsal root ganglion neurons that also respond to capsaicin (see citations in Table 2). The loss of such currents in sensory neurons dissociated from TRPV1 null mice [16,17], provides strong evidence for an obligatory role of TRPV1 in the composition of channels carrying these currents. However, there are inconsistencies in the detailed properties of the native currents which could be explained if all the current is not carried by TRPV1 homomers. The pharmacology of native i_{heat} currents is variable in capsaicin-sensitive neurons although the ligands used are not ideal for performing definitive studies. So, for example, capsazepine has been reported as a weak blocker or to have no effect (Table 2). Similarly, ruthenium red block was poor [32] or had no effect up to 100 μM [29]. Further, in isolated patches at the single channel level, there is a poor correlation between numbers of capsaicin activated and heat activated channels consistent with some heterogeneity in the native signalling units [33].

In larger capsaicin insensitive DRG neurons, inward currents activated by temperatures above 51 °C, with higher Ca^{2+} permeability, are seen [31,34]. This sub-type of larger sensory neurons express TRPV2 protein but are not immunoreactive for TRPV1 [34]. These authors demonstrate that the high threshold response is a specific current and not due to non-selective membrane or protein destruction, because the current is reversible, specifically blocked by ruthenium red and only observed in a sub-population of neurons. Comparing the properties of these currents with those evoked in HEK293 cells expressing VRL-1 (TRPV2) there are clear similarities. In addition to general similarities in the inward current properties, the temperature threshold, Ca^{2+} permeability and block by ruthenium are quantitatively almost identical in the native and recombinant current (Tables 1 and 2). Taken with the localisation of TRPV2 to these large diameter cell bodies, the evidence suggests that TRPV2 is a major component of the high threshold current in DRG. Clearly, more work is needed to understand the functional expression of TRPV2-mediated currents as a number of laboratories including our own have failed to replicate the findings of Caterina et al. [19]. Some unidentified accessory protein, which was endogenously expressed in the particular experimental protocol, is the most likely explanation. Alternatively, if as it seems possible for TRPV4 (see above and [27]), TRPV2 thermosensitivity might depend on some endogenous intracellular ligand acting as a co-agonist. If so, this might also explain the lack of response when tested in some expression systems.

8. Properties of heat-sensitive currents in mammalian thermostats

In mammals maintenance of core body temperature is achieved with the aid of multiple thermosensors present in the pre-optic anterior hypothalamus (POAH), medulla oblongata and spinal cord [35]. Extracellular recording from POAH revealed a population of warm-sensitive neurons that showed thresholds of 37 °C and firing rates above this temperature of about 5 spikes s^{-1} $°C^{-1}$. Other neurons either showed no temperature sensitivity or were cold sensitive with firing rates that declined on heating [36]. Voltage clamp

studies of the warm-sensitive neurons identified a cation current reversing at 0 mV activated by increasing temperature and cell-attached patch recording identified unitary inward currents that were about 2 pA in amplitude at the resting membrane potential [36]. As currents indicative of action potential firing were seen in these patches, this would suggest the membrane potential was less negative than −45 mV. Assuming a membrane potential of −40 mV and a reversal potential of 0 mV gives a unitary conductance of ~50 pS for this temperature-sensitive channel.

TRPV4 is expressed in the anterior hypothalamus [25] and TRPV4 currents have similar temperature sensitivity [25] to the POAH cell currents [36]. Watanabe et al. [27] found a lower threshold for TRPV4 current activation but interestingly the unitary currents through these recombinant channels had a similar conductance (59 pS) to the native POAH currents. TRPV3 also has appropriate temperature sensitivity so it would be interesting to see if this channel was expressed in hypothalamus. Direct measurement of the unitary conductance of this channel gave a value of 170–200 pS [10] but this was at +60 mV. At negative membrane potentials a unitary conductance consistent with the noise analysis derived estimate of 48 pS [9] is probable. Thus, data to date support a role for TRPV4 in transducing temperature in these hypothalamic neurons but does not exclude TRPV3.

9. Other heat-sensitive cells

The functional activation of TRPV4 expressed in vascular endothelial cells [27] suggests that this channel may have a role in local vascular responses to changes in temperature. Elevating temperature above body temperature would be expected to activate channels, causing a rise in endothelial Ca^{2+} levels. This would stimulate release of vasorelaxants resulting in local vasodilatation. Conversely, cooling could lead to vasoconstriction as the basal tonic Ca^{2+} influx through TRPV4 was reduced. This, then provides a theoretical mechanism for mediating peripheral cardiovascular responses to limb heating and cooling in mammals. It will be interesting to test this hypothesis in intact tissues.

10. Conclusions: TRPVs and endogenous heat sensation

The past year has added two new TRPV channels to the collection of heat-sensitive channels. We most likely now have the complete set of cation channels with which to fully explore the molecular basis for thermosensation in mammalian cells. We can now look forward to further work defining the properties of the recombinant channels in more detail, including the mechanism of heat sensation. This will also guide and stimulate a re-analysis of endogenous i_{heat} currents both in cells where i_{heat} has been described but also in cells such as endothelial cells and keratinocytes that had not, until now been thought of as thermosensors. Data from the study of mice in which TRPV2, TRPV3 or TRPV4 have been deleted are also eagerly awaited.

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